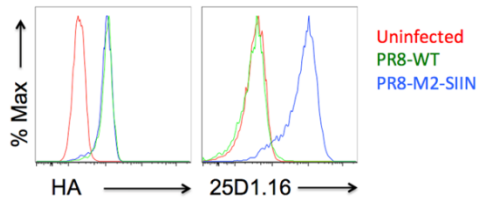
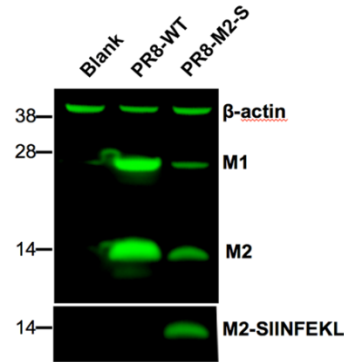
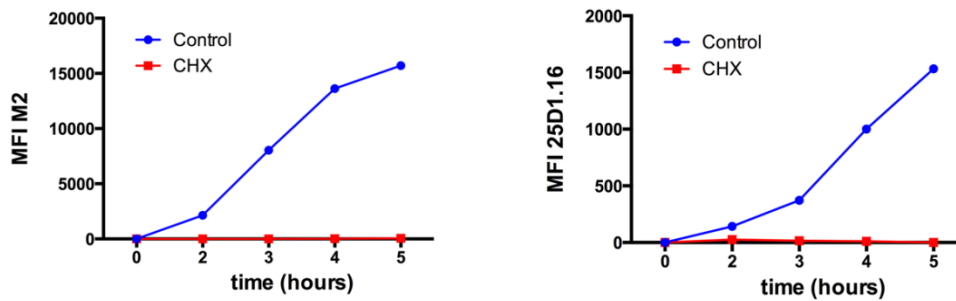
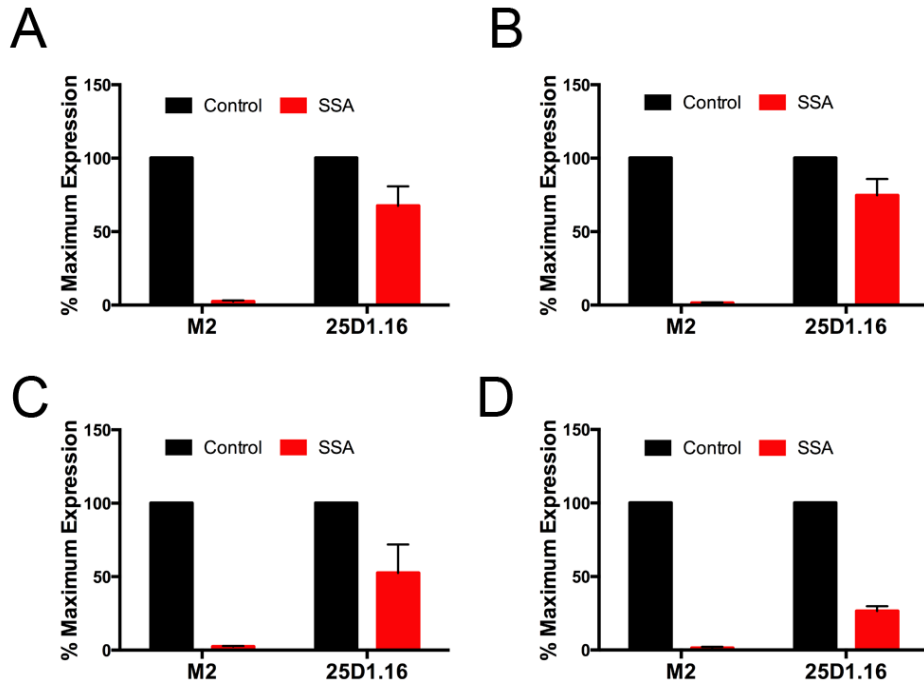


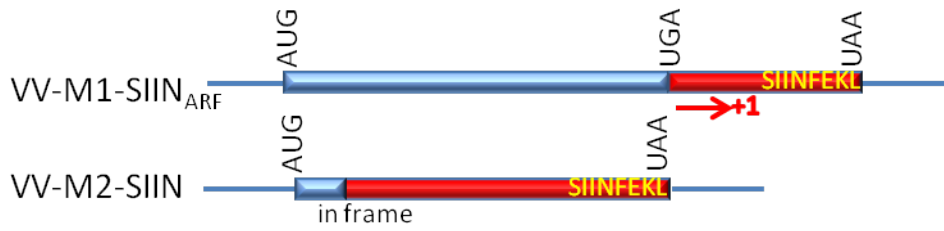
**A****B****C**

**Fig S1. Characterization of PR8-M2-SIIN and antigen presentation after infection.**

(A) Flow cytometry analysis of surface levels of HA (left panel) and K<sup>b</sup>-SIIN (right panel) in L-K<sup>b</sup> cells after PR8 or PR8-M2-SIIN infection for 12 h. (B) Immunoblot of M1, M2, SIINFEKL and  $\beta$ -actin (loading control) present in total cell lysates from L-K<sup>b</sup> cells infected with PR8 for 12 h. Uninfected cells were used as a blank control. (C) L-K<sup>b</sup> cells were infected with PR8-M2-SIIN, then CHX (25  $\mu$ g/ml) or DMSO (control) were added. At indicated times, cell-surface M2 and K<sup>b</sup>-SIINFEKL were measured by flow cytometry.



**Fig S2. CUG start codon mediated mRNA translation partially contributes to antigen peptide generation after mRNA splicing inhibition.** (A) L-K<sup>b</sup> cells were infected with PR8-M2-M72L-SIIN, then SSA or MeOH (control) was added. After 20 h infection, cell-surface M2 and K<sup>b</sup>-SIIN were analyzed by flow cytometry for the indicated times. The relative levels are represented as the percentage of the control. (B-D) L-K<sup>b</sup> cells were infected with PR8-M2-C888T-SIIN (B), PR8-M2-G1001A-SIIN (C) or PR8-M2-C888T-G1001A-SIIN (D), Cell-surface M2 and K<sup>b</sup>-SIIN were analyzed by flow cytometry.



**Fig S3.** Diagram of constructs expressed in rVVs. Blue represents M1 reading frame, red represents M2 reading frame. Note that in M1-SIIN<sub>ARF</sub> SIINFEKL is downstream of a stop codon and in the +1 reading frame. As vaccinia virus cannot splice mRNA, the SIINFEKL must be synthesized via non-standard translation.